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ESSENTIAL OIL COMPOSITION OF THREE SPECIES OF *THYMUS* GROWING WILD IN MAZANDARAN, IRAN

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ABSTRACT. The genus *Thymus* has a wide distributional range and chemical composition of the essential oils varies with geographical location of collection site, climate and other ecological factors. The essential oils of the aerial parts were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Twenty seven components were characterized in the essential oil of *T. fedtschenkoi*. The major constituents of the oil were carvacrol (69.04%), thymol (5.95%), borneol (5.21%), *p*-cymene (4.20%), bornyl acetate (2.97%) and 1,8-cineole (2.72%). Twenty two components were characterized in the essential oil of *T. trauveterri*. The major constituents of the oil were carvacrol (54.02%), thymol (9.29%), borneol (3.51%), *p*-cymene (18.64%) and *γ*-terpinene (2.97%). Twenty six components were characterized in the essential oil of *T. pubescens*. The major constituents of the oil were carvacrol (13.85%), *α*-terpineol (11.49%), thymol (10%), geraniol (9.48%), α-pinene (8.52%), *p*- cymene (7.66%), camphor (4.66%), *γ*-terpinene (3.15%) and myrcene (2.22%). Twenty four components were characterized in the essential oil of *T. fallax*. The major constituents of the oil were carvacrol (41.84%), *p*-cymene (12.18%), *α*-terpineol (11.49%), thymol (10%), *γ*-terpinene (8.68%), borneol (5.11%), geraniol (4.35%) and geranyl acetate (2.16%).

Keywords: essential oil; *Thymus fedtschenkoi; T. trauveterri; T. pubescens, T. fallax*

INTRODUCTION

The genus *Thymus* L. (*Lamiaceae*) consists of about 350 species of herbaceous perennials and subshrubs (Morales, 1986). The mediterranean region can be described as the center of the genus (Stahl-Biskup, 1991). A number of 18 *Thymus* species has been reported in flora Iranica and six of them have been known endemic (Abousaber *et al.,* 2002; Mozaffarian, 1998). *Thymus* species are commonly used as tonic,

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carminative, digestive, antitussive, expectorant and for the treatment of cold in Iranian traditional medicine. Recent studies imply that these species have strong antibacterial activities (Vila, 2002).

The Iranian popular name for the genus is "Avishan" (Rechinger, 1982). *Thymus fedtschenkoi* L. is widely distributed in Mazandaran, from sea-level up to altitudes of 1400 m, growing on sandy and siliceous soils (Ghelichnia, 2010).

Thymus species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, the leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, flavouring agents (condiment and spice), antiseptic, antitussive and carminative as well as treating colds (Alimirzaee *et al.,* 2009; Zargari, 1990).

Thymus oils and extracts are widely used in pharmaceutical, cosmetic and perfume industry as well as for the purpose of flavoring and preservation of several food products (British pharmacopoeia, 1988). Recent studies have showed that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (Sefidkon and Askari, 2002; Zargari, 1990).

Many studies on composition of essential oils from different *Thymus* species have been carried out, one of which is *T. kotschyanus*. The published results reveal that major volatile constituents obtained from the aerial parts of the plant are thymol, carvacrol, *p*-cymene, c-terpinene, b-caryophyllene, etc. (Guseinov *et al.,* 1987; Kasumov and Gadzhieva, 1980; Kulieva *et al.,* 1979; Sefidkon *et al.,* 1999).

The genus *Thymus* has made it one of the most popular plants throughout the entire world due to its volatile constituents. Therefore, there is a considerable research interest in the compositional analysis of *Thymus* essential oils obtained from the aerial parts of the plant (Vila, 2002). The essential oil substances are thymol, carvacrol, *p*-cymene, β-pinene, γ-terpinene, β-caryophyllene, 1-borneol, 1,8-cineole, etc (Rustaiyan *et al.,* 2000; Sefidkon and Askari, 2002). It is believed that a part of these activities is due to its volatile constituents.

The aim of this research paper is to determine the chemical combination of the essential oils collected aerial parts of *T. fedtschenkoi*, *T. trauveterri* ,*T. pubescens* and *T. fallax* from north of Iran, during the flowering period.

MATERIALS AND METHODS

Plant material

The aerial parts of *Thymus* species *(Thymus fedtschenkoi*, *T. trauveterri, T. pubescens and T. fallax*) were collected during flowering stage in June 2012, from mountain rangelands of Mazandaran province, in North of Iran. Voucher specimens were identified by Dr. Ziba Jamzad and deposited at the herbarium of Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

Isolation of the essential oils

After collection, the flowering aerial parts materials were shade dried at room

temperature (22-26°C) and placed in paper pockets. Samples transferred to Laboratory of Research Institute of Forests and Rangelands (RIFR), Tehran, Iran. In order to estimate the rate of essential oils, the distillation method was used (Sefidkon *et al.,* 1999). Dry plant matter were milled to a powder in an electric blender. The essential oil of all air-dried samples (100 g) was isolated by hydrodistillation for 4 h, using a Clevenger-type apparatus, according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988; Maisonneuve, 1983). The essential oil yield of samples were calculated based on dry weight, and then the oil was dried over anhydrous sodium sulfate.

Identification of compounds

The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C6-C24) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Adams, 2001). For quantification purpose, relative area percentages obtained by FID were used, without the use of correction factors.

RESULTS

The essential oil content of the dried flowering aerial parts of *T. fedtschenkoi* obtained by hydro distillation, were 1.2%, with yellow color and a distinct sharp odor. The components of the oil, the percentage of each constituent and their retention indices are summarized in *Table 1*. Twenty seven components were characterized in the essential oil. The major constituents of the oil were carvacrol (69.04%), thymol (5.95%), borneol (5.21%), *p*-cymene (4.20%), bornyl acetate (2.97%) and 1,8-cineole (2.72%). Other components were presents in amounts less than 2% (*Table 1*).

The essential oil content of the dried flowering aerial parts of *T. trauveterri,* obtained by hydro distillation, were 1.1%, with yellow color and a distinct sharp odor. The components of the oil, the percentage of each constituent and their retention indices are summarized in *Table 1*. Twenty two components were characterized in the essential oil. The major constituents of the oil were carvacrol (54.02%), thymol (9.29%), borneol (3.51%), *p*-cymene (18.64%) and γ-terpinene (2.97%). Other components were presents in amounts less than 2% (*Table 1*).

The essential oil content of the dried flowering aerial parts of *T. pubescens,* obtained by hydro distillation, were 1.2%, with yellow color and a distinct sharp odor. The components of the oil, the percentage of each constituent and their retention indices are summarized in *Table 1*. Twenty six components were characterized in the essential oil. The major constituents of the oil were carvacrol (13.85%), α-terpineol (11.49%), thymol (10%), geraniol (9.48%), α-pinene (8.52%), *p*-cymene

(7.66%), camphor (4.66%), γ- terpinene (3.15%) and myrcene (2.22%). Other components were presents in amounts less than 2% (*Table 1*).

The essential oil content of the dried flowering aerial parts of *T. fallax*, obtained by hydro distillation, were 1.1%, with yellow color and a distinct sharp odor. The components of the oil, the percentage of each constituent and their retention indices are summarized in *Table 1*. Twenty four components were characterized in the essential oil. The major constituents of the oil were carvacrol (41.84%), *p*-cymene (12.18%), α-terpineol (11.49%), thymol (10%), γ-terpinene (8.68%), borneol (5.11%), geraniol (4.35%) and geranyl acetate (2.16%). Other components were presents in amounts less than 2% (*Table 1*).

Table 1 - The composition of the essential oils (%) of four *Thymus* species in Mazandaran, Iran

No	Compound	Components (%)			
		T. fallax	*T. pubescens*	*T. trauveterri*	*T. fedtschenkoi*
1	α-Pinene	0.25	1.89	8.52	1.48
2	Camphene	1.14	1.15	4. 66	1
3	Myrcene	1.50	0.90	2.22	1.47
4	p-Cymene	4.20	18.64	7.66	12.18
5	1,8-Cineol	1.72	0.97	1.89	1.26
6	γ-Terpinene	1.59	2.97	3.15	8.68
7	Linalool	0.77	0.09	1.43	0.18
8	Geijerene	0.47	0.16	0.31	0.25
9	Camphor	0.16	-	0.13	-
10	Pinocarvone	0.28	-	-	-
11	Borneol	5.21	3.51	5.02	5.11
12	Carvacrol methyl ether	0.7	0.11	1.23	0.15
13	Geraniol	0.31	0.30	9.48	4.35
14	Bornyl acetate	2.97	-	2.62	1.11
15	Thymol	5.95	9.29	10.14	10.06
16	Carvacrol	69.04	54.02	13.85	41.84
17	Geranyl acetate	0.07	-	0.35	2.16
18	(E)-Caryophyllene	0.55	1.35	1.48	1.37
19	β-Selinene	-	-	0.12	-
20	Germacrene D	0.23	0.28	0.16	0.35
21	δ-Cadinene	0.86	1.57	5.70	0.98
22	Spathulenol	0.16	0.28	0.36	0.12
23	Elemol	0.17	-	0.19	-
24	Caryophyllene oxide	0.15	0.17	-	0.15
25	δ-Cadino	0.1	-	-	-
26	α-Terpineol	0.55	0.08	11.49	0.1
27	Thymol methyl ether	-	0.11	1.2	0.49
28	Total	99.1	97.84	88.7	94.84

DISCUSSION

In this study found thymol and carvacrol as the main components of these *Thymus* species like most of the other studies on *Thymus* spp. and both samples were rich of monoterpenes. On the other hand, the presence of p-cymene (18.64% and 12.18%) in *T. trauveterri and T. fallax* and *α*-terpineol (11.49%) in *T. pubescens* found as a distinguished marker. The results of this study on *Thymus* species collected from the North of Iran showed that oils in all of them contained the highest amount of carvacrol.

The main chemotype of essential oil were identified carvacrol, thymol, borneol in *T. fedtschenkoi* and carvacrol, thymol, *p*-cymene in *T. trauveterri* and carvacrol, thymol, *p*-cymene, geraniol, α-pinene, *α*-terpineol in *T. pubescens* and carvacrol, thymol, *p*-cymene, *γ*-terpinene in *T. fallax*. The study of on the essential oils of *T. fedtschenkoi* from another location of Iran has shown that the percentage of thymol (31.8%), carvacrol (24.3%), *p*-cymene (12.3%) and 1,8-cineole (5.8%) were in flowering stage (Abousaber *et al.,* 2002).

The study of essential oils of *T. fedtschenkoi* var. *handelii* from Turkey has shown that the main components were carvacrol and thymol and linalool, respectively (Başer *et al.*, 2001).

The study has shown that linalool (17.29%), bornyl acetate (9.19%), borneol (10.4%), thymol (9.19%) and carvacrol (6%) were also determined in *T. fedtschenkoi* var. *handelii* from Ararat mountains in Turkey (Meriçli, 1986).

The previous results showed that our oil was characterized by the presence of four dominating components: carvacrol, thymol, borneol and *p*-cymene. These four components have been previously found as constituents of most *Thymus* oils (Mc Gimpsey *et al.,* 1994; Sefidkon *et al.,* 1999; Stahl-Biskup, 1991). Comparison of the results obtained in this study with previously reported data of the *Thymus* species oils from different countries showed that the predominance of thymol, carvacrol, *p*-cymene, borneol and 1,8-cineole as main components (Sefidkon *et al.,* 1999).

In contrast with other studies, this study shows that the amount of carvacrol in the essential oil of *T. fedtschenkoi* is very high (69.04%), that this amount has not been observed in other studies. In addition the amount of α-terpineol (11.49%) in *T. pubescens* and *p*-cymene (18.64%) in *T. fedtschenkoi* was seen in this study but not seen in other studies.

Although comparison between compounds obtained from this study, and other reports, shows some similarities, but there are considerable quantitative and qualitative differences between these samples. These variations in the essential oil composition might have arisen from several differences (climatic, seasonal, geographical, and geological).

CONCLUSION

The essential oil constituents of flowering aerial parts of the aerial parts of *Thymus* species *(Thymus fedtschenkoi*, *T. trauveterri, T. pubescens* and *T. fallax* (family *Lamiaceae*), collected from different locations of Mazandaran in Iran, were studied. In conclusion, our results demonstrate variations in the qualitative and quantitative composition in the oils obtained from the aerial parts of four distinct *Thymus* species. These differences can probably be attributed to the genetic differences or different geographic or environmental conditions of the plant materials. It have been also identified a number of differences, compared with previous studies, the differences could be due to environmental conditions. The high diversity of thymus essential oil compounds that observed in this study and other studies. So, increased the possibility of selection of colonies of this plant with specific biological activity for use in the pharmaceutical industry, food, cosmetics and sanitary.

REFERENCES

Abousaber M., Hadjakhoondi, A., Shafiee, A., 2002 - Composition of the essential oil of *Thymus pubescens* Boiss. et Kotschy ex Celak and *Thymus fedtschenkoi* Ronniger from Iran. J. Essent. Oil Res., 14: 154-155.

Adams R.P.. 2001 - Quadrupole mass spectra of compounds listed in order of their retention time on DB-5. Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Co, Carol Stream, IL, USA, p. 456.

Alimirzaee P., Gohari A.R., Hajiaghaee R., Mirzaee S., Jamalifar H., Monsef-Esfahani H.R., Amin Gh., Saeidnia S, Shahverdi AR., 2009 - 1-methyl malate from *Berberis integerrima* fruits enhances the antibacterial activity of ampicillin against *Staphylococcus aureus*. Phytother. Res., 23:797-800.

Başer K.H.C., B. Demirci, N. Kirimer, F. Satil, G. Tumen, 2001 - The essential oils of *Thymus migricus* and *T. fedtschenkoi* var. *handelii* from Turkey. Flavour Fragr. J., 17, 41-45.

British Pharmacopoeia, 1988 - Vol. 2, London: HMSO, 137-138.

Ghelichnia, H., 2010 - The study of ecological properties of *Thymus* species in Mazandaran, Iran. Agriculture and Natural Research Center of Mazandaran, 150 p.

Guseinov S.Y., Kagramanova K.M., Kasumov F.Y., Akhundov R.A., 1987 - Studies on the chemical composition and on some aspects of the pharmacological action of the essential oil of *Thymus kotschyanus* Boiss. Farmakologiya i Toksikologiya, 50 (73-74).

Kasumov F.Y., Gadzhieva T.G., 1980 - Components of *Thymus kotschyanus*. Khimiya Prirodnykh Soedinenii, 1, p. 728.

Kulieva Z.T., Guseinov D.Y., Kasumov F.Y., Akhundov R.A., 1979 - Investigations of the chemical composition and some pharmacological and toxicological properties of the *Thymus kotschyanus* essential oil. Akademiya Nauk Azerbaidzhanskoi S.S.R. Doklady, 35: 87-91.

Maisonneuve S.A., 1983 - European Pharmacopoeia, Vol. 1, Sainte Ruffine, France.

Mc Gimpsey J.A., Douglas M.H., Van Klink J.W., BeauregardvD.A., Perry N.B., 1994 - Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. Flav. Fragr. J., 9: 347-352.

Meriçli F., 1986 - Volatile oils of *Thymus kotschyanus* var. *glabrescens* and *Thymus fedtschenkoi* var. *handelli*. J. Nat. Prod., 49 (5), p. 942.

Mozaffarian V., 1998 - A dictionary of Iranian plants names. Farhang Moaser Publishers, Tehran, 547-548.

Morales R., 1986 - Taxonomía de los géneros *Thymus* (excluida la sección Serpyllum) y *Thymbra* en la Península Ibérica. Ruizia 3, 1-324.

Rechinger K.H., 1982 - Flora Iranica, Vol. 152, Akademische Druck und Verlagsanstalt, Graz.

Rustaiyan A., Masoudi S., Monfared A., Kamalinejad M., Lajevardi T., Sedaghat S., Yari, M., 2000 - Volatile constituents of *of* three *Thymus species* grown wild in Iran, Planta Medica, 66: 197-198.

Sefidkon F., Jamzad Z., Yavari-Behrouz R., Nouri-Sharg D., Dabiri M., 1999 - Essential oil composition of *Thymus kotschyanus* Boiss. et Hohen from Iran. J. Essent. Oil Res., 11: 459-460.

Sefidkon F., Dabiri M., Rahimi-Bidgoly A., 1999 - The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* Boiss. & Hohen. Flav. Fragr. J., 14(6):405-408.

Sefidkon F., Askari F., 2002 - Essential oil composition of 5 *Thymus* species. Research Institute of Forests and Rangelands. Iranian J. Med. Aromat. Plants Res., 12: 29-51.

Stahl-Biskup E., 1991 - The chemical composition of *Thymus* oils: a review of the literature 1960-1989. J. Essent. Oil Res., 3: 61-82.

Vila R., 2002 - Flavonoids and further polyphenols in the genus *Thymus. In:* Thyme: The genus *Thymus*. Medicinal and Aromatic Plants-Industrial Profiles, E. Stahl-Biskup and F. Saez (Eds), p. 75, Taylor and Francis, New York, USA.

Zargari A.,1990 - Medicinal plants. Tehran University Press, Tehran, Iran, 4, p. 38 (In Persian).